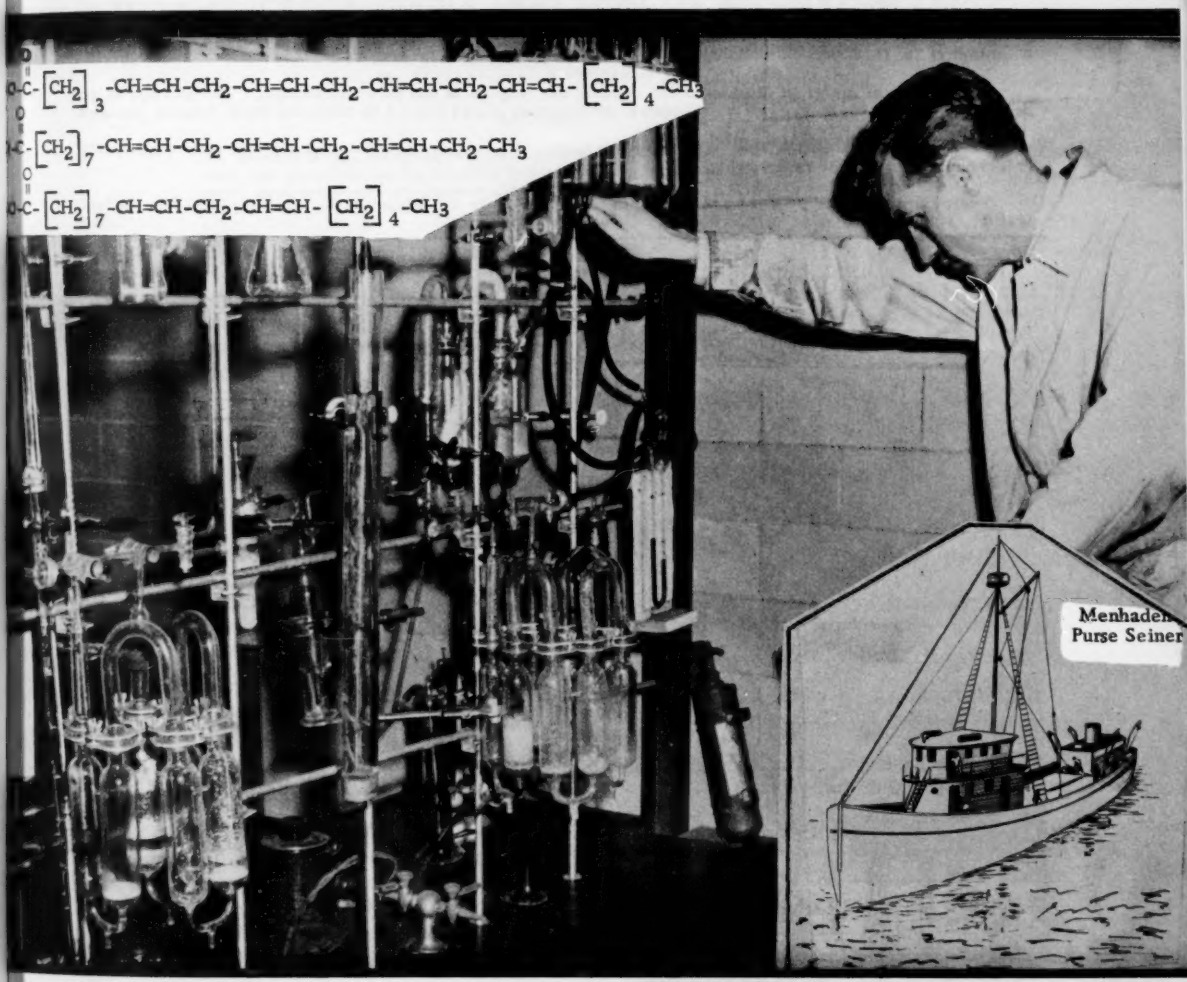
COMMERCIAL FISHERIES REVIEW



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COMMERCIAL FISHERIES REVIEW

A review of developments and news of the fishery industries prepared in the BUREAU OF COMMERCIAL FISHERIES.

Joseph Pileggi, Editor
H. M. Bearse, Assistant Editor

Mailed free to members of the fishery and allied industries. Address correspondence and requests to the: Chief, Branch of Market News, Bureau of Commercial Fisheries, U. S. Department of the Interior, Washington 25, D. C.

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CONTENTS

COVER: Fish oil research in U. S. Bureau of Commercial Fisheries technological and university research laboratories has led to the isolation of important chemical compounds for use as raw materials in the manufacture of industrial and pharmaceutical products. As production of these materials gains momentum, new and expanding markets for fish oils are opened.

COMMERCIAL FISHERIES REVIEW

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THE NONSAPONIFIABLE FRACTION OF MENHADEN OIL

William A. Mosher, Wiley H. Daniels,
Jack R. Celeste, and William H. Kelley*

ABSTRACT

Methods of separating the nonsaponifiable portion from menhaden oil were evaluated, and procedures for fractionating this portion then were investigated. In this latter work, three principal fractions were obtained: (1) squalene, (2) cholesterol and other steroids, and (3) color bodies.

INTRODUCTION

A fundamental study of the chemical identity of the components of fish-body oils was one of the primary objects of the contractual research program started by the U. S. Bureau of Commercial Fisheries. One phase of this study was to determine the nature of the components in the nonsaponifiable fraction of menhaden oil. Chemical studies on samples of the body oil of menhaden were conducted at the Chemistry Department of the University of Delaware for a period of two years.

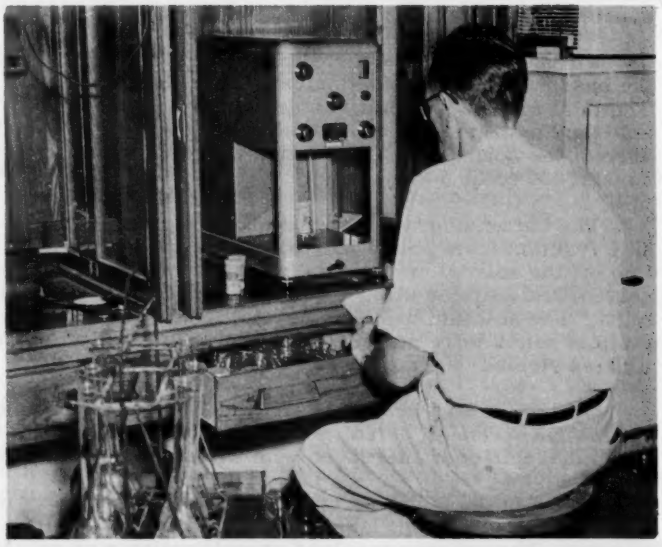

Fig. 1 - Various components of the nonsaponifiable portion of menhaden oil are quantitatively determined after selective fractionation.

EXPERIMENTAL AND RESULTS

SEPARATION OF NONSAPONIFIABLE PORTION FROM OIL: Initially, several methods of separation of the nonsaponifiable portion of the fish oils were studied. These were (1) saponification with barium hydroxide, aqueous potassium hydroxide, and methanolic and ethanolic potassium hydroxide; (2) acid hydrolysis by means of the "Twitchell" reagent, benzenestearosulfonic acid, and emulsifier that permits the acid hydrolyzing agent to contact the oil intimately; (3) enzymatic hydrolysis by the use of a lipase present in Ricinus castor bean. The same chemical products were obtained by the three methods used to separate the nonsaponifiable portion. This

*Researchers. The research reported in this paper was conducted at the University of Delaware, Department of Chemistry, under contract with the U. S. Bureau of Commercial Fisheries. It was financed by funds made available under provisions of Public Law 466, 83rd Congress, approved July 1, 1954, generally termed the Saltonstall-Kennedy Act. This article was prepared by Dr. Donald G. Snyder, Biochemist, Fishery Technological Laboratory, U. S. Bureau of Commercial Fisheries, College Park, Md., from progress reports submitted by the contractor to the Bureau.

identity of products indicates that the nonsaponifiable portion is relatively stable to acid, neutral, and strongly basic solutions.

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Of the methods studied, only the base-catalyzed hydrolysis of the fatty-acid esters present in menhaden oil was technically promising. The recovered oils from several hydrolyses were fractionally distilled and crystallized, but good isolation of compounds could not be attained. Separation by chromatography on alumina apparently was a more satisfactory and precise method.

The saponification of the oil for one hour with alcoholic potassium hydroxide gave the best results and the cleanest handling material. Several large batches of oil were saponified by this method. An average of 1.68 percent total materials unsaponified by potassium hydroxide was obtained.

FRACTIONATION OF NONSAPONIFIABLE PORTION: Solvent extractions of the unsaponifiable portion indicated that cholesterol and other sterols were present. The total steroid fraction was determined to be about 15 percent of the unsaponifiable portion of the oil, and the squalene fraction, to be about 23 percent. The balance of the nonsaponifiable portion was determined by chromatographic methods to consist of "color bodies."

The chromatographic method consisted of freeing a solution of the unsaponifiable fraction from solids by chilling and then concentrating the fraction by evaporating off the solvent on a steam bath. The ethereal extracts were dried and redissolved, and suitable aliquots were made for adsorption analyses on columns of alumina. The solution then was developed with petroleum ether. By this method, three distinct zones were noted: (1) bright yellow, (2) pink, and (3) faint yellow. Other eluates yielded varying bands of colors.

During the course of this study, it was noted that the nonsaponifiable fraction of the oil had a rancid, acrid odor entirely different from that of the whole oil. It was found that this odor increased with age owing to autoxidation.

As a result of further studies, a very satisfactory method was perfected for isolating the nonsaponifiable fraction of menhaden oil in large-scale laboratory apparatus. The process involved saponification with potassium hydroxide in water-ethanol for less than an hour. A continuous liquid-liquid extractor permitted convenient extraction of the nonsaponifiable fraction from the soap solution with ethyl ether. These large-scale saponifications yielded about 1 percent unsaponifiable material.

The steroid fraction was found to be about 90 percent cholesterol when determined by fractional crystallization from acetone and ethanol (2:3 v/v) and chromatographic adsorption on an alumina column. About 10 percent of the nonsaponifiable fraction was determined to be a concentrated fraction of color bodies. This

fraction was separated by adsorption analysis on an alumina column into three distinct oily fractions, which varied from golden yellow to red-brown.

In an effort to determine the best chromatographic method of separating the unsaponifiable fraction of menhaden oil preparatory to identifying the various fractions, numerous trials were conducted with relatively small columns, using various adsorbents and solvents. The adsorbents used were alumina, barium carbonate, silicic acid, and magnesium trisilicate. Solvents investigated with these adsorbents included petroleum ether, hexane, benzene, dichloromethane, ether, ethanol, ethyl acetate, and acetone. In many of the trials, the results obtained from chromatographic analysis with the various adsorbents and solvents could not be duplicated exactly; and in some trials, the results appeared contradictory. This lack of precision indicated that the original materials were changing in composition before or during the chromatographic procedures. Also, it was observed that fractionation of the nonsaponifiable components from menhaden oil was complicated by autoxidative changes during saponification and crystallization. These changes occurred even when special precautions were taken. The characteristic absorption spectra of several fractions were completely destroyed, for example, when the process was exposed to air. Thus, an investigation was attempted to separate chromatographically the entire nonsaponifiable fraction without the preliminary fractionation. A complete retention of spectral characteristics was obtained only when chromatographic experimental procedures were conducted under nitrogen.

Results from the initial study indicated no sharp separation of the steroid materials from the color body or carotene fraction, but the odor was reduced markedly. Squalene, however, was readily separated by this procedure. During the course of this work, it was found that a hydrocarbon and oxyginated fraction were obtained by partitioning the carotenoids between petroleum ether and methanol (90 percent).

SQUALENE: A satisfactory chromatographic technique was selected for separating the squalene from the whole nonsaponifiable fraction. The squalene was fractioned from petroleum ether on a column of alumina. The crude material was separated by the formation of hydrochlorides into three isomers that were found to be identical with squalene. The squalene was determined quantitatively in the eluates by iodometric titration procedures after chromatography on alumina. The percentage of squalene in the unsaponifiable fraction was determined to be about 5 percent.

STEROL: Attempts to isolate an impurity obtained previously in the sterol fractions were rather unsuccessful. Crude sterol fractions obtained from two recrystallizations were chromatographed on a column of alumina, and arbitrary eluant fractions were collected from benzene and methanol. Small amounts of impurity were obtained by this procedure. It was possible, however, that at least a part of the material considered as an impurity may have been squalene that was occluded in the crystalline mass of the crystallizing cholesterol. This possibility was likely, since both squalene and the impurity were eluted very early from the columns of alumina while one of the later arbitrary eluant fractions contained cholesterol of very high purity. The fraction that contained the very pure cholesterol was eluted with methanol and did not represent a 100-percent recovery of total cholesterol.

An attempt was made to determine total cholesterol in the unsaponifiable matter by precipitating gravimetrically the cholesterol with digitonin. Digitonin forms a molecular complex with cholesterol in the molecular proportion of 1 to 1. In this procedure, 100 grams of menhaden oil was saponified, and the unsaponified material was collected after drying under a hot stream of carbon dioxide. This material was dissolved in ether, and a small aliquot of the ether solution was dissolved further in ethanol. The digitonin was added next, and the resultant precipitant was dried to constant weight. By this method, the content of cholesterol in the unsaponifiable matter was determined to be somewhat more than 25 percent.

As was mentioned before, the cholesterol fraction could not be separated entirely from the color bodies or pigment fraction. At this time, other procedures were attempted to afford this separation. Columns of aluminum silicate were used with hexane, benzene, and methanol as developers. As it was determined, however, that all of the eluant fractions contained some cholesterol, the aluminum silicate was considered to be nonselective. No further work was attempted on the complete separation of the sterol and pigment fractions by chromatographic techniques.

COLOR BODIES: All further work consisted of trying to identify the pigment fractions of the unsaponifiable material of the oil. A sample of the menhaden oil was saponified, and the unsaponified matter was partitioned between petroleum ether and methanol. The petroleum ether layer was dried over anhydrous sodium sulfate, and the solvent was by a Renco solvent evaporator. The residue then was dissolved in petroleum ether and chromatographed in a column containing a 1 to 1 mixture of magnesium oxide and celite. By this method, no colored zones or bands were detected visually, but two bands were detected under ultraviolet light. These bands were eluted from the column with petroleum ether. The solvent then was removed, and the solid material was dissolved in petroleum ether as before. The spectra of these fractions indicated that more than one substance was present in each fraction.

In an attempt to isolate the individual substances in each fraction, the first fraction from above was rechromatographed on a column of alumina and developed with petroleum ether followed by a 50-percent mixture of petroleum ether and benzene. No visible results were observed when either of these solvents was used. Methanol next was used as a developer, and two bands were developed. One of these bands remained adsorbed on the column, and one eluted. The results from an analysis of the spectra of each band indicated that the materials still were not pure.

The band eluted from the column therefore was chromatographed on another column composed, in this case, of calcium hydroxide and was developed with petroleum ether. A narrow band was noted that separated from the bulk of the material and that was eluted from the column. Continued development with petroleum ether effected elution of the other band. A spectral analysis of the two bands indicated that the materials still were not pure. The band that initially was eluted from the calcium hydroxide column therefore was chromatographed in a column of 50-percent celite and magnesium and was developed with petroleum ether. One band was eluted, the solvent was removed, the residue was dissolved in n-hexane, and the visible spectrum was determined. The adsorption peaks obtained corresponded to the absorption peaks reported for alpha-carotene.

Testing the material dissolved in anhydrous chloroform with antimony trichloride resulted in the development of a blue color as is reported for alpha-carotene. The band that was eluted later from the calcium hydroxide column was treated in the same manner as was the first band that was eluted. Examination of the spectra of this material indicated absorption peaks that corresponded to peaks reported for gamma-carotene. The antimony trichloride test with this material also was positive. Thus, the presence of alpha and gamma carotene was indicated in the unsaponifiable material of menhaden oil. The second fraction that was chromatographed on the magnesium oxide-celite column and detected by ultraviolet light was not studied.

The material obtained in the methanol or hypophase during partition of the unsaponifiable matter between methanol and petroleum ether was investigated next. The solvent was removed and the residue was dissolved in petroleum ether. The solution then was chromatographed in an alumina column, with the use of petroleum ether as a developer. This step resulted in the development of six zones. The first four zones were close together and were removed physically from the column. These zones were eluted from the column with methanol, the solvent was evaporated, and the residue was dissolved in benzene. The residue dissolved in benzene was developed chromatographically on alumina. Five zones were found of varied colors.

Further development with benezene resulted in the elution of the two lower zones. The zones that remained were developed with a 20-percent acetone-benzene solution. The top zone, a yellow one, eluted but lost color on standing for several days. The second zone above split into a faintly yellow zone that remained and a yellow zone that moved rapidly with the developer. The rapidly moving zone was isolated. The spectrum obtained compared fairly well with the spectrum of xanthophyll in petrol and in hexane.

Continued development with 20-percent acetone-benzene resulted in the elution of a heavy yellow zone. No separation was obtained when this zone was rechromatographed in an alumina column with the use of benzene as a developer. When 5-percent acetone-benzene was used, however, five zones were developed. The fifth zone was eluted by further development with 5-percent acetone-benzene. The solvent was removed and replaced with n-hexane. The spectrum obtained compared fairly well with violaxanthin in petrol. The fourth zone also was eluted fairly easily with the same developer. This zone was not studied.

Further development for 2 days with the same solvent system resulted in the union of zones 3 and 2. When this united zone finally eluted, the fraction was arbitrarily split into two. Neither of these fractions was identified.

After this long period of development, the remaining zone on the column was split into three zones. The column then was extruded physically, and the three zones were separated and eluted separately with methanol. The spectra of the two lower zones corresponded very closely to that of zeaxanthin. The top zone and the various other zones obtained during the complete chromatographic procedure were not identified.

During the course of this phase of the study, it was suspected that the introduction of bulky groups into the carotenoid molecules would facilitate crystallization of the carotenoid molecules and thus afford a purer sample for chromatography. For this reason, the carotenoid molecules were acetylated. These acetylated carotenoids then were developed on alumina with various solvents, but none of the fractions obtained was crystalline in nature. Violaxanthin and xanthophyll again were indicated in certain fractions, but the absorption maximums apparently were not affected by acetylation.

At this time an attempt was made to remove the cholesterol by a direct-adsorption process utilizing "Florisil," 1/ a magnesium silicate adsorbent. This attempt was not successful.

SUMMARY

Chemical studies were conducted with samples of menhaden oil to determine the nature of the components in the nonsaponifiable fraction of the oil. Initially, three separate methods of hydrolysis were investigated to determine the best method of obtaining the nonsaponifiable fraction of the oil. Of these, alkaline hydrolysis of the oil with alcoholic potassium hydroxide was assessed as giving the best results and the cleanest handling material. By this method, nearly 2 percent of unsaponifiable material was obtained.

The unsaponified material was fractionated into three groups: (1) squalene, (2) cholesterol and other steroids, and (3) pigments. These three fractions comprise nearly 75 percent of the total nonsaponifiable fraction of the oil. The balance of the unsaponifiable material was in the form of oily substances that were not characterized.

The squalene amounted to about 10 percent of the nonsaponifiable fraction of the oil. Squalene may readily be separated from the fraction by chromatography

1/ Floridin Co., Tallahassee, Fla.

from petroleum ether on columns of alumina. The crude material was separated by the formation of hydrochlorides into three isomers that proved to be identical with ordinary squalene. The squalene was determined quantitatively by an iodometric titration procedure.

The steroid fraction amounted to about 30 percent of the nonsaponifiable matter. Results obtained by gravimetrically precipitating the cholesterol with digitonin indicate that the cholesterol comprises about 90 percent of the steroid fraction. A second sterol apparently is present as a minor constituent, but it could not be identified. Crystallization of the cholesterol from methanol resulted in an impure fraction, whereas chromatographic separation on alumina with methanol resulted in a very pure fraction. Several attempts were made to separate by chromatography the entire nonsaponifiable fraction directly without preliminary fractionation, but the cholesterol fraction could not be entirely separated from the color body or pigment fraction. Odor was reduced markedly, however, and color and the stability of color were improved by adsorptive fractionation.

The remaining nonsaponifiable portion of the oil was the color-body or pigment fraction. This fraction consists of a very complex mixture of substances of fairly high molecular weight. Most of these are the carotenes. The following were tentatively identified: alpha and gamma-carotene, zeaxanthin, violaxanthin, xanthophyll, and two oxygenated carotenes of undetermined structure. These materials can be separated by adsorptive fractionation procedures. The carotene pigments probably contribute to much of the color in menhaden oil and to the poor stability of the color.

During the course of this study, it was noted that the nonsaponifiable fraction of the oil had a rancid, acrid odor that increased with age, owing to autoxidation.

RED SNAPPER

The red snapper (Lutjanus aya) is a deep-water fish (found at depths of 20-60 fathoms) and concentrated in relatively confined areas. Snappers usually school a few feet off the sea bottom, but have been observed to surface. It is believed that they remain in one locality for considerable lengths of time. The sea floor over which the fish are found commonly consists of patches of hard limestone covered with live corals and grass. The number of such habitats is relatively small. Irregular bottom formations, such as depressions or elevations and folds, appear to be preferred habitats for red snappers. The better known red snapper fishing spots in the northern Gulf of Mexico are off Ft. Walton, Carrabelle, and Pensacola in Florida, and off the Texas coast.

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EXPERIMENTAL STUDIES TO EXTEND USES OF FISH OILS IN THE LEATHER INDUSTRY

Part 1 - Experiments with Menhaden Oil [1/]

By Victor Mattei* and W. T. Roddy**

ABSTRACT

The work reported here indicates that a satisfactory fat liquor can be made with menhaden oil. In the course of this work, a new technique for sulfating menhaden oil was developed. A public-service patent is being sought for this process.

INTRODUCTION

The leather industry requires large quantities of oils, greases, and waxes in the tanning and dressing of various kinds of leathers. Cod oil, an imported indus-

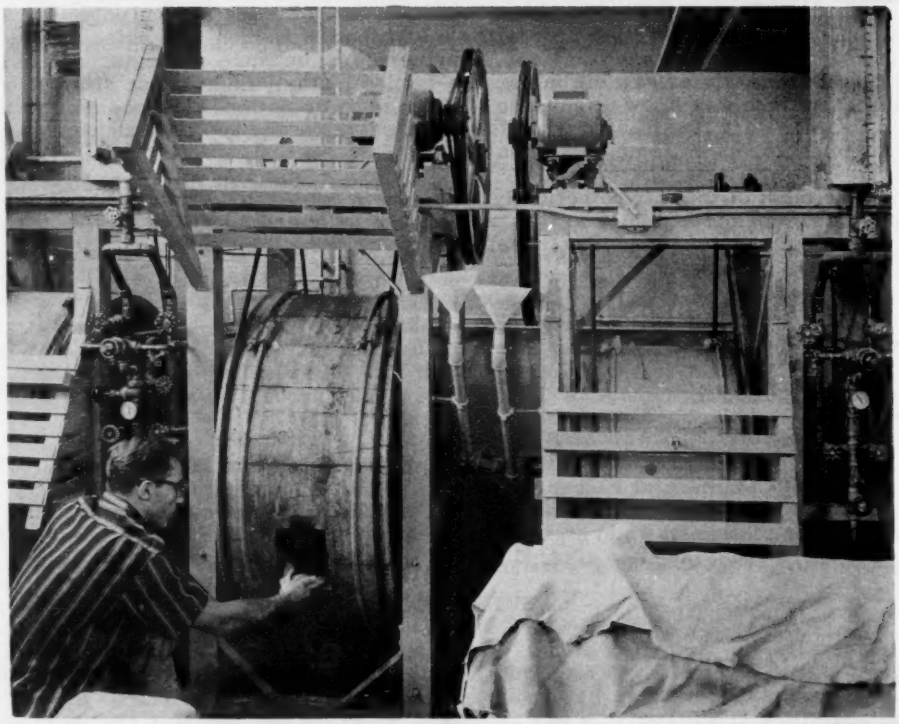

Fig. 1 - Pilot plant drums are used for fat-liquoring leather.

trial cod-liver oil not produced in this country, is the only fish oil that is being used at present in any quantity. Other oils have been tried experimentally but have not

1/ This study was supported by funds made available under the Saltonstall-Kennedy Act, U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries, awarded a contract for the study to the Tanners' Research Laboratory.

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been considered satisfactory for one reason or another. A research program was developed to determine possible value of menhaden oil, at present available in large quantities, on fat-liquoring leather. Normally, both raw and sulfated oils are used for fat-liquoring so the research was mostly concerned with sulfating menhaden oil to reduce the rate of oxidation and polymerization which have previously caused difficulties in the use of the oil. The results obtained thus far are summarized in this report.

FAT-LIQUORING

Fat-liquoring consists of treating tanned wet skins or hides with an oil-in-water emulsion. The emulsion is taken up by the fibrous leather matrix, and the emulsion breaks and deposits oil, mainly within a certain thickness in the outer portion of the leather. During the drying of the leather, the deposited oil forms a film around the fibers, giving the finished product flexibility and serviceability.

PROBLEMS IN THE USE OF MENHADEN OIL FOR FAT-LIQUORING

Many oils are capable of reducing the cohesion of the fibers, but all oils do not produce the requisite feel, temper, and firmness in the leather. Tanners considered menhaden oil undesirable because of the high rate of oxidation and polymerization. They have felt that such properties might tend to harden the surface of the leather and unduly increase firmness. Furthermore, because of the susceptability to oxidation, the filmed raw oil on chrome-tanned leather might cause spontaneous combustion. Gummy-spew might also be formed by oxidation. The possible yellowing of leather surfaces treated with fish oil might make the use of it undesirable in the manufacture of white or pastel leathers.

Since most of these hypothetically undesirable properties of the oil are associated with its chemically-unsaturated portion, the indicated approach was to reduce the amount of unsaturation. Two possible ways of doing this are by epoxidation and sulfation.

EPOXIDATION

The work on epoxidation was suggested by staff members of the Bureau's Fishery Technological Laboratory, College Park, Md. They pointed out that by partially lowering the iodine number of the oil, the product probably would be less reactive and more desirable for use in fat-liquoring.

Epoxidized menhaden oils with iodine numbers of 156 and 117 were supplied by the Bureau of Commercial Fisheries. These products have not been completely evaluated at the present time. A British Patent (1958) describes the use of epoxidized oils for oiling and waterproofing leather, indicating that possible uses exist for epoxidized menhaden and other fish oils.

SULFATION

Generally, the content of oil in a fat-liquored leather is about 5 to 6 percent, calculated on a dry basis. In fat-liquoring, sulfated oils are mixed with sufficiently large proportions of raw oils to modify the properties of the mixture. Since large quantities of sulfated oils are employed in the leather industry, the use of sulfated menhaden oil would enhance the chance of our finding new markets.

The work on the sulfation of menhaden oil first was carried out in the laboratory. The procedures developed in the laboratory then were tested on a larger scale in the pilot plant.

LABORATORY STUDIES

The laboratory studies were designed to compare, before and after 9 months' storage, the properties of leathers fat-liquored with menhaden oil, cod oil, and

neatsfoot oil. The aging of fat-liquored leather was considered necessary because of the oxidation and polymerization properties of an oil such as menhaden. The oil properties mentioned could produce a stiff leather with time as well as causing a decrease in extractable grease. This could adversely change the physical properties of the fat-liquored leathers necessitating the evaluation at the end of nine months' shelf storage. Both cod-oil and neatsfoot-oil fat liquors are presently used in the leather industry (but for different types of leather). The oxidation and polymerization properties of neatsfoot oil are different from those of cod and menhaden oil, and the characteristics of the leather produced also differ. The danger of spontaneous combustion, formation of gummy spew at high concentration of oil, and yellowing of leather surfaces exists with cod oil, though possibly to a lesser extent than with menhaden oil. For these reasons, evaluation of menhaden-oil fat-liquored leather as compared to cod-oil and neatsfoot-oil fat-liquored leathers should indicate whether menhaden oil is satisfactory for use in fat-liquoring.

SULFATION OF MENHADEN OIL: Although a sulfated menhaden oil would be a desirable product, industry personnel stated that this oil is difficult to sulfate by regular plant procedures. They pointed out that special precautions must be taken in order to prevent an extremely rapid reaction accompanied by excessive oxidation. At the Tanners' Council Laboratory, a procedure was developed for sulfating menhaden oil that reduced the hazards involved. A public-service patent is now being sought for this process.

PROCEDURE IN LABORATORY STUDIES: Chrome-tanned leather samples were fat-liquored to a 6-percent fat content on the basis of dry weight of leather. The fat-liquors consisted of mixtures of 60 parts sulfated oil and 40 parts raw oil (by weight) prepared from menhaden, cod, and neatsfoot oils.

RESULTS OF LABORATORY STUDY: The results of the laboratory study for the new and aged leathers indicated that neatsfoot-oil fat-liquored leather produced a leather that was softer to the feel[2/], was less firm, did not yellow on the surface, and showed less decrease in extractable grease after aging than did the leathers treated with either cod oil or menhaden oil. The same tests for leather quality when applied on a comparative basis between cod-oil and menhaden-oil fat-liquored leathers indicated that no large differences existed between them. No important differences were found in the strength properties of the differently fat-liquored leathers. None of the three fat-liquored test leathers produced spews. On the basis of these encouraging results, pilot-plant fat-liquoring studies were conducted to verify the laboratory findings.

PILOT-PLANT STUDIES

The pilot-plant fat-liquoring work was conducted at a commercial tannery. Four 100-pound batches of chrome-tanned hides were dyed black and fat-liquored, with the following combinations:

Test I. 60-40 mixture of sulfated menhaden oil and raw menhaden oil.
Test II. 60-40 mixture of sulfated cod oil and raw cod oil.
Test III. 60-40 mixture of sulfated menhaden oil and raw cod oil.
Test IV. Tannery control (neatsfoot-oil fat liquor).

Table 1 - Analytical Data on Sulfated Oils Used in Pilot-Plant Fat-liquoring Tests

Oil Tested	Water	Total Alkali	Acid Value	pH	Combined SO_3
	(Percent)				Percent
Sulfated menhaden oil of Test I.	25	0.52	12.7	6.2	5.6
Sulfated cod oil of Test II	25	1.3	41.0	6.5	4.7
Sulfated menhaden oil of Test III.	25	1.2	46.0	6.2	4.7

2/Neatsfoot oil is purposely used by tanners to produce a soft leather.

The sulfated menhaden oils employed in this study were prepared on a pilot-plant scale by a cooperating chemical company, using the procedure developed at the Tanners' Council Laboratory. The sulfated menhaden oil of Test III differed from that of Test I in that it was prepared to meet the specifications for the sulfated cod oil used in Test II. The analytical data on the oils are presented in table 1.

RESULTS OF PILOT-PLANT STUDY: The purpose of the pilot-plant study was to determine if a menhaden-oil fat-liquor produced a desirable leather in comparison to leather fat-liquored with the neatsfoot oil or with the cod oil now in common use. Plant evaluation showed no significant difference between cod-oil and menhaden-oil fat-liquored leathers, thus indicating that they have similar properties. As was expected, the neatsfoot-oil fat-liquored leather was less firm than were the test leathers fat-liquored with either cod-oil or menhaden-oil fat-liquors. Differences in firmness between the two test leathers treated with the fish oils were not noticeable. None of the fat-liquored leathers produced spew. The menhaden-oil fat-liquored leather had a fishy odor in the crust state, but this odor was not present in the finished leathers.

The results of the pilot-plant study confirm the earlier observation made in the laboratory that menhaden-oil fat-liquor is similar in properties to the fat-liquor of cod oil and that menhaden oil therefore will make a satisfactory fat liquor.

SUMMARY

1. The leather industry offers a potential expanded use of fish oils, particularly in fat-liquoring.

2. The process of fat-liquoring consists of treating tanned wet skins or hides with an oil-in-water emulsion, which after the leather is dried, gives the finished product flexibility and serviceability.

3. Although menhaden oil is the fish oil produced in largest quantity, the ease of oxidation and polymerization poses certain problems for fat-liquoring.

4. Two possible ways of reducing the tendency of menhaden oil to oxidize and polymerize are to epoxidize and to sulfate the oil.

5. The studies on epoxidation have not been completed, but the patent literature indicates that epoxidized oil can be used for oiling and waterproofing leather.

6. Sulfation of menhaden oil presents difficulties. The reaction is extremely rapid by regular procedures and is accompanied by excessive oxidation. A procedure developed during the present work and for which a public-service patent is being sought, however, reduces the hazards involved.

7. In laboratory studies, chrome-tanned leather was fat-liquored with mixtures of 60 parts sulfated oil and 40 parts raw oil prepared from menhaden, cod, and neatsfoot oils.

8. Results of the laboratory study indicated, as was expected, that the neatsfoot-oil fat-liquored leather produced a softer leather. There was also less decrease in extractable grease on aging than when either cod oil or menhaden oil were used. No important differences were found in the strength properties of the differently fat-liquored leathers, and none of the leathers produced spew. No significant differences were found between the cod-oil and menhaden-oil fat-liquored leathers.

9. To check on the favorable results obtained in these laboratory studies, the experiments were repeated on a pilot-plant scale. The results were the same as in the laboratory tests.

10. It is concluded that a satisfactory fat liquor can be made from menhaden oil.

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FISH OIL RESEARCH MAY SHED LIGHT ON HEART DISEASES

A research project to study the relationship of fish oil in the diet to cholesterol deposits in the circulatory system of the body has been authorized by the U. S. Department of the Interior, Bureau of Commercial Fisheries. The research is expected to contribute to the fund of information being compiled on various phases of arteriosclerosis, particularly the coronary types.

It is the unsaturated fatty acids (unstable and susceptible to chemical change) which abound in fish oil that are the center of attention. Studies already made under the Saltonstall-Kennedy Act for the improvement of domestic commercial fisheries have shown that fish oils contain a greater amount and a greater diversity of these unsaturated fatty acids than do many other food fats. The current investigations are also being made under the Saltonstall-Kennedy program.

In one experiment miniature pigs are being used to determine the deposition of cholesterol in the body. Fish oil fatty acids of known degrees of unsaturation will be fed to the test animals. Ultimately the animal will be killed and the arteries examined to evaluate the effects of the several diets employed.

Another test will be made on rats to determine which of the many fish oil fatty acids are essential to physiological welfare. One objective is attempting to determine the relationship of fish oils to metabolism and fat transport in the body, while still another is probing the properties of fish oil that may have pharmaceutical applications.

The nutritive qualities of fish in reference to heart disease and current related dietary research is explored in some detail in the July 1958 issue of the Commercial Fisheries Review, published monthly by the Bureau of Commercial Fisheries. Reprints of this article--Separate 515--are available through the Office of Information, U. S. Fish and Wildlife Service, Department of the Interior, Washington 25, D. C.

SIGNIFICANCE OF ULTRAVIOLET ABSORPTION DATA OF FISH-OIL FATTY ACIDS

By Edward H. Gruger, Jr.*

ABSTRACT

Several ultraviolet absorption characteristics exist that are peculiar to the double-bond character of polyunsaturated fatty compounds and that make ultraviolet absorption measurements a means of evaluating the degree of unsaturation of compounds derived from fish oils. Accordingly, when pure polyunsaturated fatty acids from fish oils become available as analytical standards, a practical quantitative analysis will be possible.

INTRODUCTION

Fish oils are made up largely of triglycerides that contain straight-chain fatty acid groups having both saturated and unsaturated carbon-carbon chain linkages. The unsaturated fatty acids obtained from fish oils may possess from one to six carbon-carbon double bonds per molecule. Experimental results indicate that these double bonds are separated by single methylene ($-CH_2-$) groups. That is, the unsaturated portions of the fatty acids have nonconjugated structures. An example of a nonconjugated unsaturated fatty acid is 9,12,15-octadecatrienoic acid:

$$CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH\ \ CH_2{-}CH{=}CH(CH_2)_7COOH \quad (a)$$

The absorption of ultraviolet light at certain wavelengths is characteristic of definite chemical structures. The ultraviolet absorption caused by carbon-carbon unsaturation is brought about only by a conjugated double-bond system. The fatty acids in fish oils occur naturally with nonconjugated unsaturation. For the ultraviolet absorption method of analysis to be effective with fish-oil fatty acids, the unsaturation in these acids therefore must be converted to the conjugated form. For example, the following equation (b) depicts the isomerization process of nonconjugated 9,12-octadecadienoic acid to the acid possessing conjugated unsaturation:

$$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH \xrightarrow[\text{heat}]{\text{alkali}} \quad (b)$$

$$CH_3(CH_2)4{-}CH_2{-}CH{=}CH{-}CH{=}CH(CH_2)_7COOH$$

ANALYTICAL METHOD: The double bonds in unsaturated fatty acids are conjugated by alkali-isomerization. The analytical procedure commonly employed is that of the American Oil Chemists' Society Tentative Method Cd 7-48: An 80-milligram[1/] sample of fatty material is mixed with 11 grams of 21-percent potassium hydroxide in ethylene glycol that is preheated to 180° C. (356° F.) in a suitable isomerization flask. An atmosphere of nitrogen is passed over the mixture during the entire isomerization process. The mixture is held at 180° C. for exactly 15 minutes, after which time the reaction is stopped by immediately cooling it to room temperature. The cooled mixture is diluted with an appropriate solvent to a known volume such that the concentration of isomerized material permits adquate measurement of the ultraviolet absorption spectra.

Spectral absorption peaks at 233, 268, 315, 346, and 374 millimicrons are the result of the presence within the molecules of two, three, four, five, and six conjugated carbon-carbon double bonds, respectively. From the relative heights of the absorption peaks can be calculated specific extinction coefficients. The extinction coefficients are used to determine the quantity of material present in the analyzed mixture which contribute to the particular absorptions. (This is discussed in the next section.)

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1/This figure is based on the unsaturation commonly found in commercial fish oils.

The specific extinction coefficients are calculated from the equation

$$K = \frac{A}{c \cdot 1} \qquad (c)$$

where k is the specific extinction coefficient, A is the absorbance (or optical density)

Table 1 - Specific Extinction Coefficients of Pure Natural Polyunsaturated Fatty Acid[1/]

Fatty Acid			Specific Extinction Coefficient				
Acid	No. of Carbon Atoms	No. of Double Bonds	233 mμ	268 mμ	315 mμ	346 mμ	374 mμ
Linoleic	18	2	91.6	-	-	-	-
Linolenic	18	3	47.5	90.5	-	-	-
Arachidonic	20	4	39.7	48.2	60.6	-	-
Eicosapentaenoic	20	5	39.4	41.2	82.4	87.5	-
Clupanodonic	22	5	43.5	46.0	56.9	50.4	-
Docosahexaenoic	22	6	41.7	52.2	29.6	27.7	29.3

1/ Herb and Riemenschneider, Anal. Chem. 25, 953 (1953). Isomerization in 21 percent KOH in ethylene glycol at 180° C. (356° F.) for 15 minutes under nitrogen.

at a given wavelength, c is the concentration of isomerized substances in grams per-liter, and 1 is the cell length in centimeters.

Listed in table 1 are specific extinction coefficients of pure natural polyunsaturated fatty acids. These fatty acids were prepared and studied by Herb and Riemenschneider (1953) in their work on microanalytical determinations of animal and vegetable oils at the Eastern Regional Research Laboratory of the U. S. Department of Agriculture.

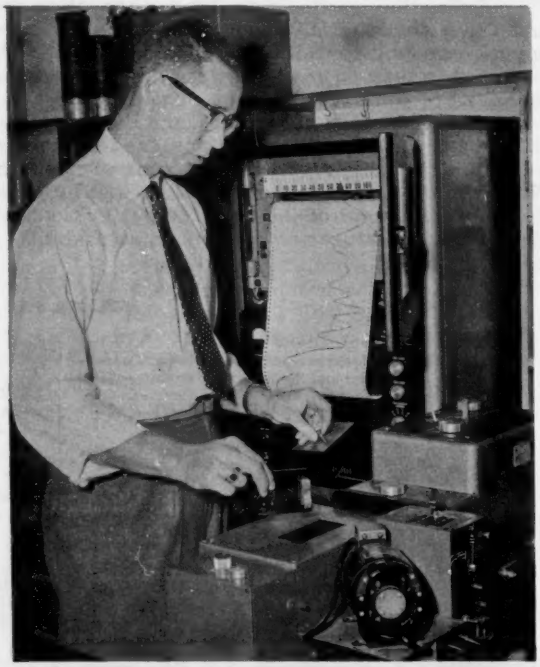

Fig. 1 - Chemist using an automatic-recording spectrophotometer to measure the ultraviolet absorption characteristics of alkali-isomerized fatty acids from fish oil.

CONSIDERATIONS: Fish oils contain fatty acid groups having from 18 to 24 carbon atoms and one to six double bonds. From table 1, it would be expected that a C_{24} fatty acid with six double bonds would have a lower extinction coefficient at 374 millimicrons than would a C_{22} fatty acid with six double bonds. This reasoning is based on the fact that a C_{22} fatty acid with five double bonds has a lower extinction coefficient at 346 millimicrons than has a C_{20} fatty acid with five double bonds. Also, this decrease in extinction coefficient can be explained by the effect of increasing chain length, which in turn increases the molecular weight. An increase in molecular weight causes a lowering of the molar concentration of double bonds for a given weight of sample. This then lowers the spectral absorption due to the double bonds and consequently lowers the specific extinction coefficient. These changes can be seen by examining equation (c).

With mixtures of highly unsaturated compounds from fish oils, such as fatty acids and fatty alcohols, the specific extinction coefficient calculated for a given wavelength is an additive value resulting from each compound in the mixture. Also, if six double bonds is the maximum number found in fish oils, there are no other ultraviolet absorptions at higher wavelengths contributing to the absorption at 374 millimicrons. One would expect, therefore, that a comparison of extinction coefficients at 374 millimicrons to the value of 29.3 (in table 1) will give a fair quantitative approximation of the content of heraenoic acids present. A pure C_{24} fatty acid of high unsaturation has not as yet been reported, thus the more complete analysis is not possible at this time.

One further matter to consider is the possibility of the existence of fatty acids of C_{20} to C_{24} chain lengths having only two and three carbon-carbon double bonds. Until the contrary has been unquestionably proven and reported, complete fatty acid analysis by ultraviolet absorption will not be possible.

APPLICATIONS: The ultraviolet absorption data are valuable as a means of determining the success of methods of separating fish-oil fatty acids or their derivatives. The data also can be used to determine the effect of storage conditions on the high degrees of unsaturation; that is, whether or not storage treatment has affected the pentaene and hexaene content.

Extinction coefficients, from absorption data of the type described above, are used in a set of simultaneous equations for solving quantitatively the percentage of each compound contributing to the particular absorptions. For quantitative analyses of this type to be accurate, however, pure compounds (the pure fatty acids in the case of fish oils) must be available for use as reference standards. Contract work being carried out by Dr. Orville Privett at the Hormel Institute, University of Minnesota, Austin, Minn., is designed to prepare the necessary "standard" fatty acids and to analyze the major commercial fish oils for their fatty acid composition. When this contract is completed, it will be possible to obtain a practical quantitative measurement of the relative proportions of the different fatty acids of various degrees of unsaturation present in all fish oils by the use of ultraviolet absorption measurements.

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VARIATION IN PHYSICAL AND CHEMICAL CHARACTERISTICS OF HERRING, MENHADEN, SALMON, AND TUNA OILS

Raymond O. Simmons*

ABSTRACT

Refractive index, iodine number, free fatty acid, saponification number, nonsaponifiable matter, stearine fraction, and Gardner color index were determined for herring, menhaden, salmon, and tuna oils. The data for menhaden oil are given for the various geographical areas along the East and Gulf coasts ranging from Long Island to the Mexican border.

INTRODUCTION

Processing industrial products from fish is an extensive industry. During 1956, in the menhaden industry alone, for example, over 2 billion menhaden were reduced to fish meal, solubles, and oil.

An adequate domestic market exists for the fish meal and solubles as components of commercial mixed feeds for poultry and swine, but the domestic demand for fish oils has declined during the last several years. One of the reasons for this decline was the preconceived concept that commercially-available fish-body oils varied considerably in physical and chemical characteristics.

The purpose of this research project was to determine the normal variation in physical and chemical characteristics of fish oils produced in the United States and Alaska. This study is a part of the over-all research program on fish oils initiated by the U. S. Bureau of Commercial Fisheries. The anticipated practical result is to extend the market for fish oils through a better knowledge of their physical and chemical properties.

EXPERIMENTAL

During the 1955 and 1956 season, samples of fish-body oils were analyzed for refractive index, iodine number, content of free fatty acid, saponification number, content of nonsaponifiable matter, and Gardner color number. During the 1956 and 1957 season these same analyses were made. In addition, the oils were separated into a stearine-oil fraction and a winterized-oil fraction, and the relative amounts were determined of these fractions. Also, the refractive index and iodine numbers were determined for these fractions.

A Bausch and Lomb Precision Refractometer was used to determine refractive index, and the Gardner color number of the oils was determined with the 1953 series Gardner color standards for liquids. All iodine values were determined by the Wijs method using a reaction time of 1 hour. Commonly-accepted procedures as outlined in the Official and Tentative Methods of the American Oil Chemists' Society were used for the other determinations. Stearine was determined by a defined winterizing process that consisted of stepwise lowering the temperature of the oil to 5° C. and separating the solid phase and the liquid phase by centrifugation.

A total of 126 menhaden and 14 herring body oils and 12 tuna and 12 salmon cannery byproduct oils were analyzed. The menhaden samples were received from plants located on the Atlantic and Gulf of Mexico Coasts, extending from Port Monmouth, N. J., to Port Arthur, Tex.; the tuna samples came from California, and the herring and the salmon samples came from Alaska.

*The research reported in this paper was conducted at North Carolina State College, Department of Chemistry, under contract with the U. S. Fish and Wildlife Service. It was financed by funds made available under provisions of Public Law 466, 83rd Congress, approved July 1, 1954, generally termed the Saltonstall-Kennedy Act. This article was prepared by Dr. Donald G. Snyder, Biochemist, Fishery Technological Laboratory, College Park, Md., from progress reports submitted by the contractor to the Service.

Table 1 - Some Physical and Chemical Properties of Various Fractions of Fish Oils Collected from Different Geographical Areas

Area	Type of Oil	Refractive Index				Iodine Number				Free Fatty Acid				Saponification Number				Nonsaponifiable Matter				Stearine Fraction				Gardner Color Index			
		No. of Samples	High	Low	Median	No. of Samples	High	Low	Median	No. of Samples	High	Low	Median	No. of Samples	High	Low	Median	No. of Samples	High	Low	Median	No. of Samples	High	Low	Median	No. Samples	High	Low	Median
											(Percent)				(Percent)				(Percent)				(Percent)						
Northern New Jersey and Long Island	Menhaden:																												
	Whole	18	1.4724	1.4698	1.4706	18	181.5	172.1	176.6	18	2.57	0.47	0.76	18	198.3	192.6	192.1	14	1.69	0.64	0.77	16	46.1	18.0	36.2	19	12	10	11
	Winterized1/	10	1.4722	1.4711	1.4712	10	194.3	179.2	187.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	10	1.4760	1.4678	1.4684	10	171.3	156.4	161.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
South Jersey and Delaware	Whole	20	1.4810	1.4689	1.4711	20	190.8	156.6	182.6	20	2.88	0.58	1.40	20	198.3	190.4	192.8	18	1.06	0.68	0.82	20	61.0	9.0	31.7	20	12	10	11
	Winterized	14	1.4735	1.4690	1.4732	14	197.1	166.8	189.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	14	1.4714	1.4651	1.4688	14	173.7	134.4	163.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chesapeake Bay	Whole	5	1.4712	1.4683	1.4695	5	175.3	159.8	169.2	5	2.73	1.33	2.11	5	194.8	191.8	193.7	3	1.30	0.91	0.97	6	61.7	36.2	39.9	6	12	11	12
	Winterized	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North and South Carolina	Whole	14	1.4699	1.4664	1.4687	14	174.0	150.5	162.6	12	3.57	0.67	1.70	12	198.2	190.4	192.6	12	2.89	0.53	1.17	14	94.2	28.2	54.2	12	13	9	12
	Winterized	7	1.4695	1.4701	1.4709	7	179.1	161.0	175.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	7	1.4689	1.4662	1.4681	7	162.9	141.4	157.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
East Coast of Florida	Whole	3	1.4680	1.4669	1.4679	3	158.6	150.0	157.5	3	2.94	1.07	2.30	3	196.5	193.3	193.8	3	1.29	0.75	1.34	3	62.8	48.7	61.5	3	14	10	11
	Winterized	3	1.4694	1.4684	1.4693	3	168.2	161.7	167.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	3	1.4674	1.4654	1.4672	3	150.9	136.4	149.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
East of Mississippi Delta	Whole	49	1.4705	1.4662	1.4673	51	182.2	140.1	152.5	51	74.20	0.77	1.70	51	201.4	180.0	195.9	40	1.35	0.13	0.92	49	76.9	19.2	46.2	51	black	10	12
	Winterized	31	1.4722	1.4673	1.4688	31	191.5	148.5	164.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	31	1.4707	1.4638	1.4663	31	168.8	123.9	139.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
West of delta to Mexican border	Whole	15	1.4706	1.4668	1.4674	15	165.2	150.7	152.7	15	3.31	1.00	1.76	15	197.5	195.1	196.4	13	1.32	0.59	0.93	15	68.0	22.4	42.8	14	12	11	11
	Winterized	12	1.4699	1.4681	1.4690	12	175.2	140.0	158.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	12	1.4664	1.4567	1.4650	12	145.8	123.2	132.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Combined data	Whole	114	1.4810	1.4662	1.4697	126	190.8	140.1	162.6	124	74.20	0.47	1.70	134	201.4	162.6	193.7	103	2.89	0.13	0.93	123	94.2	9.0	42.8	124	black	9	11
	Winterized	77	1.4735	1.4673	1.4701	77	197.1	140.0	171.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	77	1.4714	1.4567	1.4677	77	173.7	123.2	153.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
California	Tuna:																												
	Whole	12	1.4758	1.4732	1.4749	12	193.8	163.7	186.8	8	4.06	0.68	3.47	4	198.1	196.2	194.5	1	1.33	1.33	1.33	11	71.8	13.8	42.3	8	black	brownish red	dark brownish red
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alaska	Herring:																												
	Whole	14	1.4672	1.4645	1.4657	14	172.8	123.1	133.5	14	3.31	0.97	2.05	14	195.2	186.2	188.3	13	1.74	0.77	1.01	14	72.4	36.3	42.0	12	12	7	12
	Winterized	12	1.4678	1.4648	1.4665	12	155.2	120.6	135.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	12	1.4666	1.4638	1.4649	12	146.4	109.4	126.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alaska	Salmon:																												
	Whole	12	1.4707	1.4681	1.4698	12	175.1	150.8	166.6	12	3.48	1.14	2.06	12	197.5	194.9	195.7	10	1.18	0.77	1.00	12	38.5	2.6	3.0	12	13	11	12
	Winterized	8	1.4709	1.4680	1.4691	8	167.4	141.9	152.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stearine	8	1.4693	1.4668	1.4675	8	162.8	94.1	110.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1/Winterized-oil fraction; stearine-oil fraction.

RESULTS

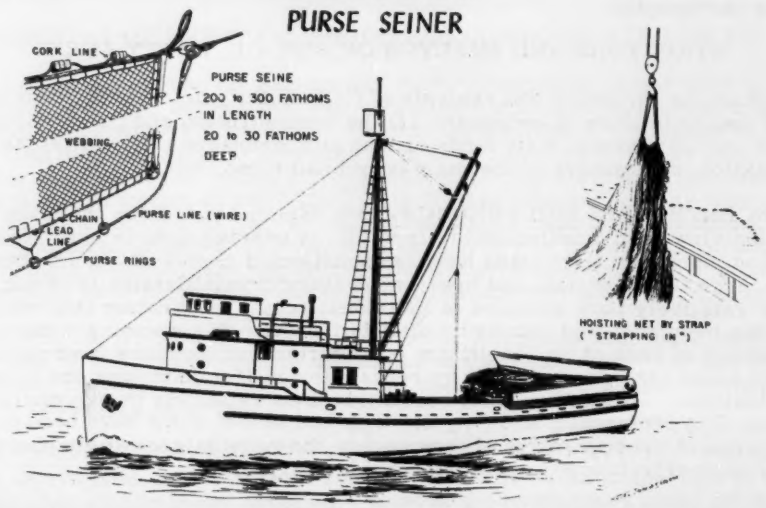
In Table 1 are presented the data obtained in the present work. In addition to the data reported here, data on analysis of oils from individual menhaden reduction plants and data on time of catch of fish from which these oils were produced have been analyzed statistically by a variance technique at the Department of Experimental Statistics of North Carolina State College at Raleigh. At present, these analyses of the physical and chemical characteristics of the fish body oils are being correlated with the many processing variables associated with the different lots of oil and will be reported later.

WEST COAST SARDINE AND TUNA PURSE SEINER

In the Pacific Coast tuna fisheries, the purse seiners or netters are next to tuna clippers in importance. Purse seiners are not as large as the clippers and have a smaller cruising radius and smaller cargo capacity. They were originally designed for sardine and mackerel fishing and usually pursue these species during the fall. Larger purse seiners may fish tuna the year-around and generally catch the same species as the clippers.

Sardine and Tuna

PURSE SEINER



The purse seiners use a large net to encircle the schools of fish. The nets generally are about 1,800 feet long and 180 feet deep and generally cost about $30,000 each.

CHEMICAL AND NUTRITIONAL STUDIES ON FISH OILS[1/]

By O. S. Privett,* J. R. Chipault,* H. Schlenk,** and W. O. Lundberg***

ABSTRACT

This paper reports progress on the following four projects: (1) determination of the structure of fish-oil fatty acids and development of analytical techniques for determination of the fatty-acid composition of fish oils, (2) study of the chemistry of odor-producing compounds in fish oils, (3) study of the chemical reactions of fish-oil fatty acids, and (4) study of the nutritional effects of fish oils.

INTRODUCTION

The Hormel Institute, a research branch of the University of Minnesota Graduate School, currently is conducting four projects in the research program established by the U. S. Bureau of Commercial Fisheries with funds made available by the Saltonstall-Kennedy Act.

The objectives and the early stages of the work in three of these projects were described previously (Lundberg 1957). They are concerned with (1) determination of the structure of fish-oil fatty acids and development of analytical techniques for the determination of the fatty-acid composition of fish oils, (2) study of the chemistry of odor-producing compounds in fish oils, and (3) study of chemical reactions of fish-oil fatty acids.

Since our last report was made, a fourth project, which is concerned with the nutritional effects of fish oils, has been started. Progress in each of these four projects since the time of the last publication in this journal is summarized in the following paragraphs.

STRUCTURE AND ANALYSIS OF FISH-OIL FATTY ACIDS

Work on the structure and analysis of fish-oil fatty acids in the past year has involved two main lines of endeavor: (1) the concentration and purification of the principal polyunsaturated fatty acids of tuna and menhaden oils and (2) the complete determination of structure of the main polyunsaturated fatty acids.

CONCENTRATION AND PURIFICATION: The more tedious task, the isolation of pure individual polyunsaturated fatty acids, is nearing completion. Methyl esters of tuna and menhaden fatty acids have been subjected repeatedly to fractional distillation, urea fractionation, and low-temperature crystallization to obtain concentrates of relatively pure samples of individual acids. Separation into various chain lengths has been achieved mainly by distillation through a spinning-band column. Fractionation of each chain length into concentrates of the more important polyunsaturated acids has been achieved by repeated urea fractionations and fractional crystallizations. By these means, concentrates or relatively pure samples of C_{22} hexaenoic, C_{20} pentaenoic, and C_{18} and C_{16} tetraenoic acids have been prepared. In the course of the separation of these acids, the materials have been characterized by alkali-isomerization and chromatographic analyses.

Although C_{22} pentaenoic acid appears to be another important polyunsaturated constituent of some fish oils, a suitable concentrate of this acid from either menhaden or tuna oil has not been obtained. Other fish oils, therefore, are being investigated as possible sources of this acid.

STRUCTURE ANALYSIS: Considerable effort has been devoted to developing a technique for determining the locations of double bonds in polyunsaturated fish-oil

1/ This study was supported by funds made available under the Saltonstall-Kennedy Act through a contract with the U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries.

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fatty acids. A permanganate-periodate oxidation technique, developed in another laboratory for less unsaturated fatty acids, has been modified satisfactorily for the structure analysis of highly unsaturated fatty acids of fish oils.

A portion of quite pure methyl docosahexaenoate prepared from tuna oil was subjected to such structure analysis. The iodine value of this sample was 441.5 (theoretical 444.7). The purity of the material was established further by chromatographic analysis. By means of the permanganate-periodate oxidation technique, it was demonstrated unequivocally that this is a 4, 7, 10, 13, 16, 19, docosahexaenoate.

For the first time, it recently was reported by another laboratory that a docosahexaenoate of this structure is present in pilchard oil. The fact that this acid now has been shown also to be present in tuna oil is of interest from two points of view: first, apparently this is the only docosahexaenoic acid that occurs in appreciable quantities in ordinary fish oils; and second, the acid found in fish oils evidently is identical with that obtained from land animals.

Only a small amount of work was done on the phase of the project concerned with developing analytical techniques for the analysis of fatty acid composition of fish oils. It is planned, however, that in the near future, when additional quantities of the pure polyunsaturated fatty acids become available, the characteristics of the individual acids in alkali isomerization will be determined, and spectral constants for fish-oil analysis will be established. On the basis of work done during the past year, another possible technique for analysis of the fatty acid composition of oils has come to light, involving a combination of distillation, ozonolysis, and paper chromatography of the oxidation productions of ozonolysis. This method of analysis will also be investigated because, if successful, it will yield more complete results than does the alkali-isomerization method.

CHEMISTRY OF THE ODOR-PRODUCING COMPOUNDS IN FISH OILS

Much of the work done on odoriferous materials in fish oil has been concentrated on the isolation, separation, and identification of carbonyl compounds because such compounds previously have been found to be involved in the oxidative deterioration of fish oils and because they also have been reported to be the cause, at least in part, for the "reverted" odors of vegetable oils. The distillates obtained by vacuum-steam deodorization at 100°-120° C. (212°-248° F.) and also by flushing the oxidized oil with nitrogen at 35°-40° C. (95°-104° F.) have been examined. Column and paper chromatography have been used extensively in the separation and identification of the 2,4-dinitrophenylhydrazine derivatives of the carbonyl compounds. These methods have been supplemented by ultraviolet and infra-red spectrometry.

MONOCARBONYL COMPOUNDS: Monocarbonyl compounds that have been shown to be present in the steam-volatile distillates are formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, and hexaldehyde. Unsaturated aldehydes also have been detected.

UNKNOWN 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES: The monocarbonyl derivatives, however, represent a relatively small proportion of the total 2,4-dinitrophenylhydrazones obtained. Most of the hydrazones could not be chromatographed on paper by any of a number of methods that were tried. Most of the nonmigrating compounds gave blue to violet colors when treated with strong alkali, suggesting that they are derivatives of the Greek letter "ALPHA" α-dicarbonyl compounds, but some of the nonmigrating fractions remain unchanged on treatment with alkali.

From ultraviolet spectra, it has been determined that the unknown 2,4-dinitrophenylhydrazone derivatives are quite different, in some cases, from those of known mono- and dicarbonyl derivatives. Efforts therefore are being made to

separate and identify such carbonyl derivatives in a different manner. The volatile compounds from fish oils have been neutralized with sodium hydroxide and subjected to steam distillation to remove nonacidic compounds. These, in turn, have been treated with silver oxide to oxidize the aldehydes to acids, which again have been separated from the rest of the compounds by neutralization and steam distillation. Preliminary examination by paper chromatography has indicated the probable presence of acetic, propionic, and suberic or azelaic acids in the acidic fraction. Some mono- and dibasic acids that have not been identified have been detected in the acid fraction formed by oxidation with silver oxide.

These studies show that a variety of volatile, saturated and unsaturated, monocarbonyl and dicarbonyl compounds contribute to the odor of oxidized fish oils. Earlier studies showed that nitrogen compounds also contribute to the odor. Although the steam distillates from the oxidized oils have strong, pungent, disagreeable odors, they do not resemble the odor of the oxidized oil, and attempts to reproduce the odor of fish oil by diluting the distillates with bland mineral oil have not been successful. Thus, the compounds causing the characteristic "fishy" odor are altered or destroyed during steam deodorization.

The volatile compounds removed from oxidized fish oil by bubbling nitrogen through the oil at room temperature have therefore been collected by various devices, including passage of the nitrogen and accompanying volatile compounds through cold traps, 2,4-dinitrophenylhydrazine solutions, and charcoal. It has not been possible to deodorize completely fish oils in this manner, and only small amounts of volatile compounds have been collected. The eluate from the charcoal trap, however, has been found to contain the characteristic fishy odor. The fact that not all of the odor is removed by passage of the gases through 2,4-dinitrophenylhydrazine suggests that other compounds may be as important as are carbonyl compounds in the characteristic odor of fish oil.

A major part of the characteristic fishy odor produced by oxidation, resides, in extremely small amount, in the more volatile fractions. Our future efforts will be directed toward the fractionation, isolation, and identification of this material.

CHEMICAL REACTIONS OF FISH-OIL FATTY ACIDS

The aim in the study of the chemical reactions of fish-oil fatty acids is to prepare new derivatives from the fatty acids occurring in fish oils, mainly by taking advantage of their unsaturation. The work has involved a study of thiourea reactions, halogen reactions, and fractionation by means of sulfur dioxide.

THIOUREA REACTIONS: The incidental finding mentioned in the previous report in this journal--that thiourea reacts with hydroperoxides of autoxidized fatty esters--has been investigated further. In menhaden oil, peroxide values up to 500 can be reduced to about 20 by treatment with a solution of thiourea in methanol at 0° C. (32° F.) or at room temperature. By this simple procedure peroxides are converted into unsaturated hydroxy acids, while unsaturated components that have not been autoxidized remain unchanged.

By using purified hydroperoxides of methyl oleate and linoleate, we found that two peroxidic groups react with one thiourea molecule. In the course of the reaction, the latter is converted into amino-imino-methane-sulfinic acid. Under these mild conditions, between 90 and 95 percent of the peroxidic groups are reduced, but the residual peroxides are not eliminated by repeating the reaction. Apparently, different types of peroxides are present, or rearrangement takes place in the purified hydroperoxides to prevent part of them from reacting with thiourea. The resulting sulfinic acid, and to some extent thiourea itself, is not stable in solvents at 50° to 70° C. (122°-158° F.). When the reaction is forced to completeness at elevated temperatures, sulfurized compounds are formed from the secondary products and from the highly unsaturated esters.

HALOGEN REACTIONS: Although halogenated fatty acids often are used to characterize unsaturated lipides, they have found little practical application. Fish oil would be an excellent source of polyhalogenated lipides. Instead of following the usual procedures by which halogens are introduced into unsaturated lipides, we used reactions of chlorinated or brominated carbon compounds. The reactions of double bonds with dihalocarbenes, which so far had been studied only with olefins of low molecular weight, have been applied to olefins derived from fatty acids and to unsaturated fatty alcohols and fatty acid esters. Chloroform or bromoform, when reacted with potassium t-butylate, yields potassium salt and dichloro- or dibromo-carbene. The latter are highly reactive, short-lived reactants that add to double bonds to form derivatives of dichloro- or dibromocyclopropane. Octadecene-1, for example, is converted in good yield into n-hexadecyl-2,2-dichlorocyclopropane. The same reaction can be carried out with oleyl alcohol or oleic acid esters where the double bonds are in the 9,10 position. Both double bonds of methyl linoleate can be reacted, and 50 percent of the double bonds of menhaden-oil fatty acid methyl esters have been converted into dichlorocyclopropane rings. Crude menhaden oil, as received for the purposes of this project, was reacted without drying it or subjecting it to preparatory processing. By varying the conditions, we could regulate the extent of the reaction, and glyceride mixtures were obtained in which 20 to 50 percent of the double bonds were consumed.

The halogen atoms in the cyclopropane ring are remarkably resistant to chemical agents. In accordance with their lowered level of unsaturation, the products either autoxidize much more slowly than do the original oils, or do not autoxidize at all. They are oily liquids like the unsaturated materials from which they have been derived but are heavier than water.

FRACTIONATION IN SO_2: Fish oils contain a considerable amount of saturated fatty acids, and any efficient and economical process of refinement that will eliminate them may enhance the commercial value of the oils. Crystallization, with or without solvent, or liquid-extraction processes are the conventional means for lipide fractionation. Separations are particularly difficult with fish oils because of the great variety of chain lengths occurring in them and the various degrees of unsaturation of their components. For several decades, petroleum chemists have used sulfur dioxide to refine hydrocarbons, achieving thereby separation of the saturated from the unsaturated cyclic and hetero-atomic hydrocarbons. The literature reveals little on fractionation of lipides by means of SO_2, and a study of this method of fractionation therefore has been undertaken.2/

The curves of solubility versus temperature of the common fatty acids and esters in SO_2 were determined, which then made possible the selection of optimal temperatures for fractional extractions of mixtures. The preparative experiments were carried out with menhaden oil or distilled fractions of it, for which we had detained analyses from previous work. Sulfur dioxide proved to be at least as efficient as were the common organic solvents for such fractionations. Menhaden-oil fatty acid esters, I. V. 151.5, could be separated by a one-step procedure into a saturated fraction, I. V. 1.2, and an unsaturated fraction, I. V. 179. Methyl esters of the C_{18} fraction of menhaden-oil esters, I. V. 105, were split into fractions having I. V. 1.4 (m.p. 36.5°-37° C.) and I. V. 134, respectively. As expected, traces of oleate, linoleate, and linolenate could be demonstrated by paper chromatography in the saturated fraction, but stearate could not be found in the unsaturated one. The amount of stearate had been assayed previously by paper chromatography to be about 25 percent, while in this separation, 22 percent was isolated. In another experiment, crude menhaden oil, I. V. 177, was separated into glyceride fractions having I. V. 112 and 210.

The structures of the lipides are not changed by the treatment with SO_2, and recoveries are virtually 100 percent. Sulfur dioxide is not flammable, and it has a

2/A detailed report on this investigation will be published in the near future.

boiling point of -10° C. (14° F.). It can be handled at low temperatures like any organic solvent, and it is easily recovered. For the laboratory investigator, its pungent odor makes efficient ventilation necessary. The use of SO_2 on a large scale in processing and at the same time as a refrigerant has been mastered with hydrocarbons. The use of it with lipides should not present any greater problem.

NUTRITIONAL EFFECTS OF FISH OILS

Our study on the nutritional effects of fish oils was started in the summer of 1957. It consists of three phases: (1) a determination of whether fish oils contain significant quantities of essential fatty acids, (2) a study of the effects of the ingestion of fish oils in relation to atherosclerosis, and (3) an investigation of nutritive factors in the minor constituents of fish oils.

ESSENTIAL FATTY ACIDS IN FISH OILS: When rats are fed a fat-free diet, their growth is greatly retarded, and certain other deficiency symptoms develop, including scaly skin and loss of hair. These symptons are relieved by the addition to the diet of certain fatty acids or fatty esters, especially those of linoleic and arachidonic acids. Linolenic acid restores growth but does not alleviate the dermal symptoms. Fatty acids that are effective, partially or wholly, in relieving the symptoms are termed "essential fatty acids" (EFA).

The common belief, apparently based on little if any critical experimental work, is that fish oils contain very little essential fatty acids. The complete absence of essential fatty acid activity in fish oils would be surprising, and wholly unanticipated, on the basis of the results of structure studies of fish-oil fatty acids such as those described above.

Several commercially-important fish oils are being fed to EFA-deficient rats, and the effects of these fish oils on growth and deficiency symptoms are being observed. Concentrates of several individual fatty acids occurring in fish oils are also being fed to EFA-deficient rats, and the effects of these fatty acids are being noted. Should it develop that some of the individual fatty acids exhibit a high degree of EFA activity but that the fish oil themselves are lacking in this quality, an investigation will be made to ascertain what is causing the inhibition of the EFA activity in the natural oils.

In a preliminary study, the methyl esters of tuna-oil acids were prepared by conventional methods and separated into fractions consisting of methyl esters of 16, 18, 20, and 22 carbon atoms. These fractions then were fed to EFA-deficient rats. All of the fractions restored growth, but to date, they have not alleviated the dermal symptoms.

EFFECT OF FISH OILS AND FISH-OIL FATTY ACIDS IN ATHEROSCLEROSIS: Atherosclerosis is the basic cause of more than half of the human deaths occurring in the United States. Recent work has tended to link the degree of saturation of ingested fat with the deposition of cholesterol in the arteries. It has been reported that unsaturated vegetable oils that contain large amounts of linoleic acid lower blood cholesterol in animals and humans when incorporated in the diet. Since the polyunsaturated fatty acids of fish oils are considerably more unsaturated than are those of the common vegetable oils, it appears feasible to investigate their effect on blood levels of cholesterol and on other blood lipides.

Miniature pigs and rats will be used as the experimental animals. The pigs will be fed a special diet designed to elevate the amount of cholesterol in the blood and then will be fed diets containing representative fish oils. The results of such fish-oil supplementation will be compared with the effects produced by vegetable oils. Similar experiments will be conducted in which hypercholesteremic rats will be fed concentrates of individual fish-oil fatty acid esters. The experiments with rats are already under way, but results are not yet available.

NUTRITIVE VALUE OF MINOR CONSTITUENTS OF FISH OILS: It has been suggested that the unsaponifiable fractions of fish oils contain nutritionally important minor constituents in addition to vitamins A and D. In experiments to test this possibility, control rats will be fed synthetic diets in which all the fat consists of resynthesized glycerides from purified fatty acids free from unsaponifiables. This basal diet will be supplemented for experimental groups of rats with fish-oil unsaponifiables. The effects of such diets on growth, reproduction, lactation, and other physiological activity will be noted.

This third phase of the program will not be studied until some time in the second year of the project. It will be looked upon as secondary to the other two phases. Possibly only a preliminary investigation can be made.

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FISH-OIL RESEARCH AT THE SEATTLE FISHERY TECHNOLOGICAL LABORATORY

By Phillip A. Hart

Fish-oil research at the Seattle Technological Laboratory has been directed primarily toward utilization of the unique features of unsaturation found in fish oils. (This article appeared in Commercial Fisheries Review, April 1958, and is now available as Sep. No. 508.)

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PROGRESS ON STUDIES IN UTILIZATION OF FISH-OIL DERIVATIVES IN ORE FLOTATION

By S. R. B. Cooke

This paper describes the flotation process, the mechanism of collection, and the flotation of iron ore with fish-oil derivatives as collectors. It summarizes experimental findings to date and outlines future work. (This article appeared in Commercial Fisheries Review, January 1958, and is now available as Sep. No. 499.)

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REVIEW OF PROGRESS ON OXIDATIVE DETERIORATION IN FISH AND FISHERY PRODUCTS

By Maurice E. Stansby* and W. D. Brown**

ABSTRACT

A review of the history and accomplishments of a cooperative research program, jointly sponsored by the University of California and the Bureau of Commercial Fisheries, is presented. Oxidative deterioration in (1) extracted fish oils and (2) in the tissues of fish, is being studied in terms of autoxidative and enzymatic mechanisms. Principal findings to date include (1) analyses of conditions affecting the efficacy of antioxidants, (2) catalytic effect of hematin compounds on the oxidation of and rancidification of fish oils in situ and after extraction, (3) effects of lack of reducing vitamins in the meat upon the oxidative discoloration of the meat during the canning process, (4) carbonyl-amine reaction and coupling plays a relatively minor part in the browning or "rusting" of fish and (5) comparative rates of oxidation of different fish meals were shown to be correlated directly with the relative contents of hematin compounds. A discussion of research now under way is also presented.

INTRODUCTION

A cooperative program concerned with the oxidative deterioration that occurs in fish and fishery products has been under way since September 1955 between the Food Technology Department and the Institute of Marine Resources, both of the University of California, and the Seattle Fishery Technological Laboratory of the U. S. Bureau of Commercial Fisheries (Brown 1956, Stansby 1957). The purpose of the present report is to review the accomplishments of this program and to outline briefly the nature of the current work.

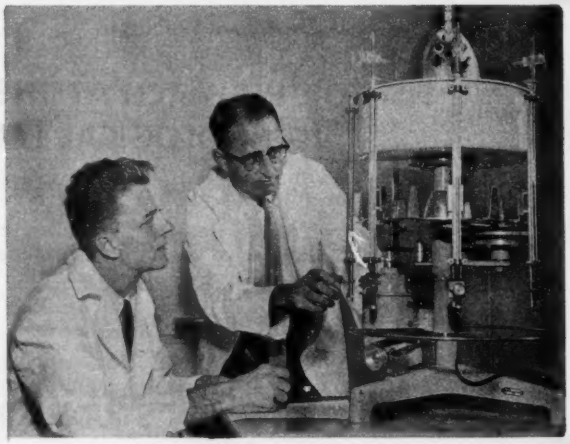

Fig. 1 - Operation of Warburg equipment for measurement of oxygen uptake by fish oils.

That portion of this program being carried out by the Institute of Marine Resources in the Food Technology Department at Berkeley is concerned primarily with the mechanism of oxidation in extracted fish oils. Dr. H. S. Olcott of the University of California is the project leader. He is assisted in the research by Dr. Edwin J. Kuta, Bureau of Commercial Fisheries chemist, and by two part-time Bureau of Commercial Fisheries physical science aides, Miss Esther Edery and Miss Carol DeJong.

That portion of the program at the Food Technology Department at Davis is concerned with oxidative deterioration occurring in the tissue of fish. Dr. A. L. Tappel of the University is the project leader. He is assisted by Dr. W. Duane Brown, Bureau chemist, and Mr. Michael Gumbmann, part-time chemist.

When the program first was started, in September 1955, it was carried out at Davis. The work began as a general preliminary survey of the mechanism of oxidation of the components (mainly oils and pigments) of fish tissue. Later, in July 1956, the presently-divided arrangement was made wherein the work on mechanism

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of oxidation in extracted oils was set up as a related but independent project for work under the then newly-established Institute of Marine Resources at Berkeley.

PRINCIPAL FINDINGS OF PROGRAM

Some of the most significant findings deal with the mechanism of oxidation in extracted fish oils. Important observations have been made (1) regarding conditions for applying antioxidants to such systems and (2) regarding the role of such factors as the amount of free fatty acid in the oil. Since these results are discussed in another report, no further mention of them will be made here.

HEMATIN CATALYSIS: The first outstanding finding from this program was the conclusive demonstration, previously shown for meat and other meat foods and now shown for fish, that the oxidation of oil in the meat, which results in rancidity, is very greatly accelerated by the presence of hematin compounds (Brown, Venolia, Tappel, and Stansby 1957). This catalytic effect was brought out strikingly in experiments in which the amount of oxygen adsorbed by cubes of dark meat and of light meat of fish was measured in a Warburg apparatus. The dark meat contains most of the hematin compounds. In these experiments, the light meat of such species of fish as salmon and tuna absorbed no measurable amount of oxygen, whereas the dark meat absorbed a very considerable amount.

These results were confirmed in other experiments in which model systems containing purified fatty acids and proteins found in fish were tested for adsorption of oxygen. Again, it was demonstrated clearly that the presence of hematin compounds increased the rate of oxygen adsorption many fold.

The content of hematin in a number of species of fish was measured. Halibut, ocean perch, trout, and cod contained between 0.1 and 0.4 x 10^{-5} M. Rockfish and lingcod contained 0.5 x 10^{-5} M; pilchard, 5.4 x 10^{-5} M; and tuna, 8.5 x 10^{-5} M. This order of arrangement of species is, in a general way, in the order of increasing susceptibility to oxidation, indicating that the content of hematin compounds is a major factor in determining the rate of development of rancidity in fish.

It was shown that during oxidation of the oil in the meat of fish, the hematin compounds decrease and are chemically altered. Thus, in one experiment (Brown et al., 1957), the concentration of hematin compound decreased during the course of the oxidation from 2.1 x 10^{-5} M to 1.0 x 10^{-5} M in the light meat, and from 67.2 x 10^{-5} M to 49.6 x 10^{-5} M in the dark meat. At the same time, the spectral adsorption curves of an aqueous extract of the samples before and after oxidation showed a change in maxima from 542 to 500 millimicrons in the one case, and from 578 to 630 millimicrons in the other, indicating a transformation of oxyhemoglobin or oxymyoglobin to methemoglobin or metmyoglobin.

TUNA PIGMENT CHANGES: The work on hematin catalysis of oil oxidation in fish tissue helped clarify an indirectly-related problem involving changes in hematin pigments in tuna, which affect color and marketability of canned tuna. Normally, the color of raw tuna changes during precooking from an indefinite gray to a light pink. This change in color is considered desirable. Some occasional batches of tuna, upon being precooked, do not become pink, however, but change to a variety of colors ranging from a greenish gray to shades of orange, tan, or brown. Such tuna are described as "green" tuna, and if the color is extreme, the fish are considered to be unmarketable. In some cases, the discoloration is accompanied by changes in texture, odor, and flavor. Before the present program was started, nothing was known about the chemistry of the changes in the pigments of tuna causing these changes in color.

Brown and Tappel (1957) now have shown that the pink color that normally develops in precooked tuna is due to hemochromes whose non-heme constitutent is either denatured globin or nicotinamide, or probably both. Brown, Tappel, and Ol-

cott (1958) have shown that the off-color (so-called "green" tuna) was caused by the presence of a hemichrome pigment that can be transformed back into the pink hemochrome by treatment with suitable reducing agents. A more rapid change occurs with the use of sodium hydrosulfite as reducing agent, and a slower change occurs with the use of ascorbic acid. In some lots of tuna, the restoration of the pink color is enhanced by the use of nicotinamide with the reducing agent. These reactions might be employed to ensure retention of proper color in the meat of fish during commercial canning of tuna.

FISH MEAL: In initial survey experiments, Brown and coworkers (1957) used samples of freeze-dried fish to simulate ideally-dried fish meal. They found that the rate of oxidation of such samples varied greatly, depending upon the part of the meat used and upon the species of fish. Thus the dark meat from freeze-dried pink salmon oxidized nearly 9 times as fast as did the light meat from this species, and a freeze-dried sample of pilchard oxidized over 300 times as fast as did one from cod.

Experiments in which antioxidants were added to commercial fish meals showed that use of BHA or BHT reduced the rate of oxidation to less than one-quarter the rate for untreated samples and that use of Santoquin (not yet approved by Food and Drug Administration) reduced the rate to less than one-eighth that of untreated samples.

PROTEIN-OIL REACTION: The mechanism of browning such as occurs in fish meat and in the rusting of frozen fish as a result of polymerization, oxidation, and the carbonyl-amine reaction has been investigated (Stansby 1957). The carbonyl-amine reaction has been found to play a minor role in such browning (Venolia and Tappel 1958).

CURRENT RESEARCH

The current research deals with (1) investigation of oxidative reaction mechanism in extracted fish oils, (2) investigation of enzymatic oxidation in the tissue of fish, and (3) further investigation of the alternation of pigment in tuna.

The investigation of oxidative reaction mechanism in extracted oils considers such factors as the effect upon oxidation rates of the presence of natural antioxidants and of contaminating heavy metals.

The investigation on enzymatic oxidation considers the enzymatic oxidation of unsaturated lipides, carbohydrate metabolism, and the tricarboxylic acid cycle in fish tissue. Each of these programs is discussed in separate reports, and accordingly, will not be discussed further here.

Additional investigation of green tuna is continuing as samples become available. Most samples of green tuna are of the type that can be reversibly changed back to the normal pink color by treatment with a reducing substance such as sodium hydrosulfite. Apparently, however, some lots of tuna are "green" because of some quite different pigment reaction, and these may not be reversibly reduced to the normal pink color. Samples of this type are rare; so far, only one has been found.

Even for those samples that can be reversibly altered back to the pink color, it is not entirely clear as to what factors in the handling of the fish determine whether use of standard canning procedures will result in green or in normal color in the precooked and canned product. In other words, do green tuna result from some factor before the fish are caught--for example, presence of some special feed--or do they result from some handling conditions, and if so, what are these conditions? Samples of tuna of known history are being obtained, and observations on effects on greening are being made. As sufficient samples for which handling history can be correlated with development of greening are obtained, the chemistry of pigment changes will be investigated with relation to this history.

DISCUSSION

The success of this program in obtaining results of practical value demonstrates the importance of a basic approach in undertaking research in the field of fishery technology. The initial planning in this program was based upon a rather theoretical investigation of factors influencing oxidative changes occurring in fish tissue. It was presumed that the first application of this basic research would be in the control of rancidity. The possibility of applying such findings to the problem of green tuna was not even considered when the program was started.

In a similar way, it is probable that work now at early stages of development on enzymatic changes and oxidative mechanisms in extracted oils may lead to applications not even conceived of at present.

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WATER PISTOL TECHNIQUE USED TO ANAESTHETIZE FISH

A new water pistol technique, developed by two American scientists, involves an anaesthetic called "M. S. 222." A sea-water solution of this drug is sprayed over a fish thrashing on the end of the line, stunning it within a minute. The technique was developed to capture sharks and rays for use in scientific studies, but these findings might also be useful to anglers who catch fish for food or exhibition. The drug has no harmful effect on the fish, and does not spoil the flavor.

The drug, an amethan-sulphonate compound, is sprayed over the fish by means of a water pistol, rubber bulb syringe, or small pump-type handsprayer. Within 15 seconds the M. S. 222 solution begins to take effect and, as a rule, even a 400-pound shark is anaesthetized in a minute or less. The fish return to consciousness in 5 to 30 minutes after being re-immersed in water, depending on their size and the amount of spray they received (Irish Fishing and Fish Trades Gazette, August 17, 1957).

REVIEW OF BASIC RESEARCH ON OXIDATIVE ENZYMES IN FISH TISSUE [1/]

W. Duane Brown,* Michael R. Gumbmann,* A. L. Tappel,** and M. E. Stansby***

ABSTRACT

Basic research on oxidative enzymes in fish tissue now has been undertaken to lay a sound foundation for effective applied research.

INTRODUCTION

Some aspects of the collaborative research program between the Department of Food Technology of the University of California and the Seattle Fishery Technological Laboratory of the U. S. Bureau of Commercial Fisheries have been described in the previous paper in this series (Stansby and Brown 1958). The present report deals specifically with one of the newer phases of investigation; namely, a study of the oxidative enzymes of fish. Knowledge of such enzymes has most direct application to two closely related areas of interest to fishery technology; (1) the nature and properties of the enzymes that survive the death of the fish and (2) the enzymes and pathways of intermediary metabolism in fish.

Fig. 1 - Measuring activity of a glycolytic enzyme by spectrophotometric assay.

A knowledge of the nature and properties of surviving enzymes is needed because of the possibility that these catalysts may bring about certain biochemical transformations, such as the oxidation of carbohydrate intermediates in fishery products that are held under refrigeration. Such transformations could be either detrimental or beneficial to the final product. In either case, an understanding of them is a prerequisite to the control of them.

A knowledge of the intermediary metabolism of fish would provide an understanding of the details of enzymic reactions involved in the synthesis and breakdown of proteins, fats, and carbohydrates. A fundamental investigation of these pathways is essential. Because of the many enzymes involved and the detail required in such a study, it is neither brief nor simple. A thorough understanding of the chemistry of fish metabolism, however, would be of great value because it would afford a basis of understanding and application to problems found in fields of investigation such as fish nutrition and commercial handling, processing, and preservation. An example pertinent to this discussion is that an understanding of fish metabolism is required in the study of surviving enzymes.

1/ This study was supported by funds made available under the Saltonstall-Kennedy Act through a contract with the U. S. Department of the Interior, Fish and Wildlife Service.

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Our initial research was directed specifically at a study of the surviving enzymes of fish. Preliminary studies were aimed at identifying such enzymes in several species of frozen fish, including yellowfin, albacore, and skipjack tuna, king salmon, ocean perch, and black rockfish. It was apparent in the early stages of this study, however, that knowledge of the fundamental pathways of metabolism in live fish would contribute greatly to a study of surviving enzymes. Consequently, this portion of the research has been suspended, pending completion of certain basic studies on intermediary metabolism. The remainder of this report will deal more specifically with the investigations of the enzymes of intermediary metabolism of fish, with emphasis on oxidative enzymes.

Suitable test fish for use in the fundamental enzymic studies have to fulfill certain requirements, including continuous availability and ease of maintenance in aquaria in a size suitable for laboratory manipulations. Members of the minnow family (Cyprinidae), particularly the common carp, fulfill these requirements and have been used in these studies. In addition to the routine use of the test fish, extensions have been and will continue to be made to various commercial food fishes by using various species of them as test animals for the study of any enzyme or reaction judged to be of particular importance, or simply by comparing various enzymic activities in commercial fish with those of our test carp.

PRINCIPAL FINDINGS

An examination of the literature revealed little in the way of information about oxidative enzymes in fish. Reports that had appeared were widely scattered, and no compilation of the existing material had been made. An extensive review of the literature therefore was completed and now is being written. This material will be published shortly.

Although little information is available about intermediary metabolism in fish, the literature contains an extensive biochemical background dealing with metabolic pathways in other animals as well as in bacteria and in plants. It appeared likely that the pathways occurring in fish would most closely resemble those found in other animals. Our initial approach, therefore, was to study certain selected areas known to be of great importance in animal metabolism, using the techniques and enzymic assays developed by other investigators in this general area. Fish are directly compared to mammals; any differences revealed by the study can be explored more intensively to gain a thorough knowledge of pathways peculiar to aquatic animals. The research to date on this problem has been concentrated on the two general areas of carbohydrate metabolism and fatty acid metabolism. The general plan of research in these studies has been to assay for activities of specific enzymes known to be catalysts in the metabolic pathways that were of interest. In general, the assay methods used for all the various enzymes follow the same type of procedure and involve some measure of the rate of the enzyme-catalyzed reaction. Since the majority of enzymes of interest are oxidative, for example, oxygen uptake could be measured in a system that contained substrate, enzyme preparation, and any necessary cofactors, all maintained in a suitably-controlled enviroment.

CARBOHYDRATE METABOLISM: Three main pathways in carbohydrate metabolism are of particular importance: (1) glycolysis, (2) the hexose monophosphate shunt, and (3) the tricarboxylic acid cycle. In each pathway, multienzyme systems catalyze a sequence of reactions resulting in the transformation of a given metobolite to chemical energy and products. Activities of many of the enzymes of these pathways have been measured in our preparations, which usually were homogenates of fish tissue.

Glycolysis refers to several reactions in an organism that are catalyzed by a multienzyme system and that result in the breakdown of starch or glycogen to pyruvic acid, with the accompanying production of compounds rich in energy. The

pyruvate formed may be transformed into lactic acid as is done in animal muscle. Additionally, it may enter into the tricarboxylic acid cycle (see below) after having been changed to an activated two-carbon form. In the over-all process of glycolysis, a large number of individual enzymic reactions are involved. In our fish preparations, assays have been made and activity found for the following glycolytic enzymes: lactic acid dehydrogenase, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, aldolase, and two phosphohexoisomerases; namely phosphomannose isomerase and phosphoglucose isomerase. The presence of these representative enzymes indicates that glycolysis is functioning in fish.

Glycolysis is generally considered to be the main pathway for the breakdown of monosaccharides to pyruvic acid. It is now well recognized, however, than an alternative pathway called the hexose monophosphate shunt exists in some organisms. It was of interest to know if this pathway functioned in fish tissue; assays for two of its enzymes therefore were made: glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. Activities of these enzymes were found, indicating that this shunt pathway functions in fish. Additional research will tell us the relative contribution to carbohydrate metabolism made by these two alternate routes.

The tricarboxylic acid cycle is of prime importance not only to carbohydrate metabolism but to fat and protein metabolism as well, since it provides a means for the various products of metabolism to be oxidized to carbon dioxide and water. This unique and versatile cycle unites glucolysis, fatty acid oxidation, and amino acid breakdown to one final oxidative mechanism. It occurs in a large number of organisms, including animals, microorganisms, and higher plants. In our research, it has been studied in more detail than has glycolysis. A majority of the enzymes known to be involved in this cycle have been found to be present in fish. In some cases, the products formed by the enzyme reactions have been measured, and the effects of various activators and inhibitors have been studied. The enzymes of the tricarboxylic acid cycle investigated to date are condensing enzyme, aconitase, isocitric dehydrogenase, succinoxidase, fumarase, and malic dehydrogenase. The evidence obtained so far in this study indicates that this cycle in fish is similar to that in other animals.

FATTY ACID OXIDATION: Considerable attention has been paid in the past to the fact that many fish contain relatively large amounts of highly unsaturated fatty acids. The significance of these unique fatty acids is well recognized from the commercial standpoint, owing to their desirability, for example, in fish-oil fractions for some purposes; or to their undesirability, for example, in contributing to rancidity in fresh or manufactured fish products and heating in fish meals. Unfortunately, little or no research has been undertaken to elucidate, at the enzyme level in fish, the biochemical basis of the synthesis and oxidation of these fatty acids.

Our initial experiments in this area have been patterned after the research of investigators who used mammalian tissue. In general, this involved preparing a cell-free fish-liver homogenate, which was fractionated by differential centrifugation into various particulate fractions. The enzyme-containing fraction (mitochondria) then was added to a system containing fatty acid and various cofactors and activators, and the consumption of oxygen was measured manometrically. Mitochondria were used as an enzyme source because most of the activity is localized in these particles; additionally, their use enables the development of a more defined system, since soluble substances are washed away, and furthermore, any information gained is at the more basic cellular component level.

The multienzyme system causing the oxidation of fatty acids in fish tissue has been studied in detail. The requirements for the system have been defined, its properties outlined, and the effect of numerous inhibitors and activators on the system observed. The ability of the system to utilize a considerable number of fatty acids has been ascertained. The fatty acids used as substrates include butyric,

octanoic, palmitic, linoleic, linolenic, arachidonic, and a fish-oil fatty acid fraction. In general, the behavior of this system resembles that observed in mammals, but there are some differences in detail.

CURRENT RESEARCH

Current research in this phase of the program is in two areas: (1) tricarboxylic acid cycle and (2) fatty acid oxidation and synthesis. The nature of the research in both these areas is primarily an extension of the work outlined above.

In the case of the tricarboxylic acid cycle, a continuation of the study will include assays for those members of the cycle not yet demonstrated in fish, as well as detailed fundamental studies of specific enzymes or reactions of particular importance. In this connection it may be pointed out that one of the enzymes of this cycle, succinoxidase, is of great interest, since it is one of the more stable enzymes and would be a surviving enzyme in refrigerated or frozen fish.

In the near future, the investigation of oxidation of the highly unsaturated fatty acids will be intensified, because these are of such great importance in fish. We propose an investigation on the synthesis of these unsaturated fatty acids by fish. This particular problem has great general interest as well, since the mechanism of biosynthesis of highly unsaturated fatty acids in any organism is not well defined.

DISCUSSION

The information gathered to date on this portion of our investigation has been purposely presented in summary fashion. Much data have been accumulated; to present even a portion of the information in detail, however, would make this report too unwieldy to serve its purpose. The detailed results of these studies therefore will be presented for publication to a biochemical journal.

As was suggested in the introduction to this paper, the most immediate application of these studies may well have to do with enzymic reactions that occur after death of the fish and that affect the desired final product. As a hypothetical example, use might be made of some surviving enzyme, for example, succinoxidase, to act as an oxygen scavenger in a packaged frozen product and thus remove traces of oxygen that might otherwise have undesirable effects on the color or flavor of the product.

It cannot be stressed too strongly that in order to develop any application, it is necessary to understand the enzymes of fish at a basic biochemical level. Our fundamental studies of certain of these enzymes are designed to provide such an understanding and thereby to lay the basis for sound applied research.

LITERATURE CITED

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1958. Review of Progress on the Program on Oxidative Deterioration in Fish and Fishery Products. (See p. 24 of this issue.)

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USE OF FISH OIL IN LEATHER TANNING

The staff of the Tanners' Council Research Laboratory at the University of Cincinnati has been investigating the value of fish oils for the lubrication of leather since 1955. This research was performed under contract for the U.S. Bureau of Commercial Fisheries and financed by funds provided by the Saltonstall-Kennedy Act of 1954. The research was successfully concluded July 1958.

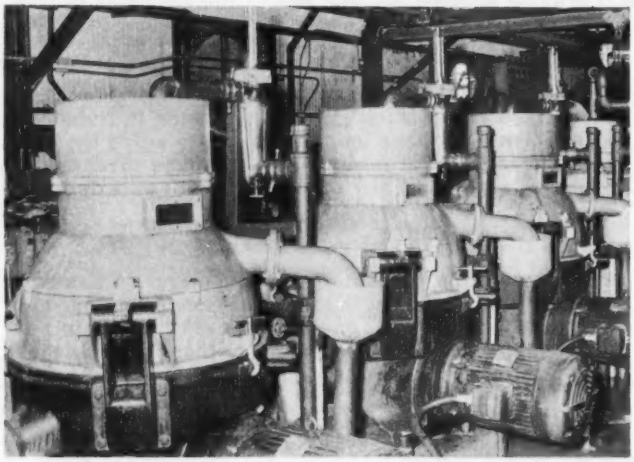

Fig. 1 - Oil Separators of the type used in fish oil production.

Further results of this contractual research, not presented in the article appearing in this supplement, indicate that satisfactory fatliquors also can be made from the oil of ocean perch, herring, and salmon--as well as menhaden. These results, as well as detailed presentation of all results obtained from this study, have been presented in a series of three technical articles prepared by Victor Mattie and W. T. Roddy of the Tanners' Council Research Laboratory. These articles are entitled "The Use of Fish Oils for Fatliquoring Leather. Part I. Menhaden Oil and Cod Oil Fatliquors; Part II. Sulfation of Fish Oils; and Part III. Menhaden Oil, Perch Oil, Herring Oil, and Salmon Oil Fatliquors." These articles will be published in the Journal of the American Leather Chemists' Association.

Reprints of these articles when available will be sent to the more than 400 members of the Tanners' Council--thus insuring optimum utilization of information obtained.